**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

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| Serial No.: | **10/004,432** | | |
| Applicants: | **Shau-Chi CHI** | TC/A.U.: | **1648** |
| Filed: | **December 6, 2001** | Examiner: | **Laurie A Scheiner** |
| Title: | **AN IMMORTAL CELL LINE DERIVED FROM GROUPER *EPINEPHELUS COIOIDES* AND ITS APPLICATIONS THEREIN** | **Customer No.:** | *23639* PATENT TRADEMARK OFFICE |
| Docket No.: | **SC7040694001 (formerly 39734-176754)** | | |

**DECLARATION OF DR. KJERSTI GRAVINGEN PURSUANT TO 37 C.F.R. § 1.132**

I, Dr. Kjersti Gravingen, hereby declare:

1. I am a researcher in the field of fish diseases and vaccine development. I am currently employed by Pharmaq AS in Norway. Pharmaq AS is the exclusive licensee of the GF-1 cell line having an ATCC deposit No. of PTA-859. Pharmaq AS is located in Skøyen, N-0213, Oslo, Norway.

2. In connection with my research in developing vaccines for immunizing susceptible fish against infection by Infectious Pancreatic Necrosis Virus (IPNV), I have studied the propagation of IPNV in the GF-1 and CHSE-214 cell lines. CHSE-214 cell line is an immortal fish cell line derived from Chinook salmon embryonic cells.

3. The "Materials and Methods" I used are summarized as follows:

*(A) Materials:*

(i) The CHSE-214 cell line is grown in a growth medium containing EMEM (Sigma M7278, FBS (Sigma); L-glutamin (Sigma G7513); and Gentamicin (Sigma G1397).

(ii) The GF-1 cell line is grown in a growth medium containing L-15 (Sigma L5520); FBS (Sigma); L-glutamin (Sigma G7513); and Gentamicin(Sigma G1397).

*(b) Methods*

The GF-1 and CHSE-214 cell lines were cultivated and passed 3 times on the growth medium containing serum before seeding of 13 cell culture flasks with each cell line. The GF-1 cells were seeded with a density of $3 \times 10^4$ C/cm$^2$ and CHSE with a density of $4 \times 10^4$ C/cm$^2$. Two flasks from each of the four groups were trypsinated and counted 3, 4, 5, 6 and 7 days after seeding. The cell counts 7 days after cell seeding were used to calculate the virus input for the remaining three flasks within each group. The infection was performed by replacing of the growth medium with fresh medium containing 2% of FBS and IPNV corresponding to a MOI of 0.1. The CHSE-214 and GF-1 flasks were incubated 3 and 5 days at 15$^0$C before sampling, respectively. The titration was performed on plates prepared from CHSE-214 cell cultures with serum. Samples from CHSE-214 cells were titrated on fresh material, whereas the GF-1 samples were frozen before titration.

4. The results of my studies are summarized as follows:

*(A) Cell growth promotion test*

The average doubling time for the CHSE-214 cells were 2.2 days (figure 1). The average doubling time for the GF-1 cells were 1.2 days (figure 2).

**Figure 1.** Cell growth promotion test on CHSE-214 cells with Sigma serum in 75 $cm^2$ cell culture flasks. Two flasks were counted at each time point.

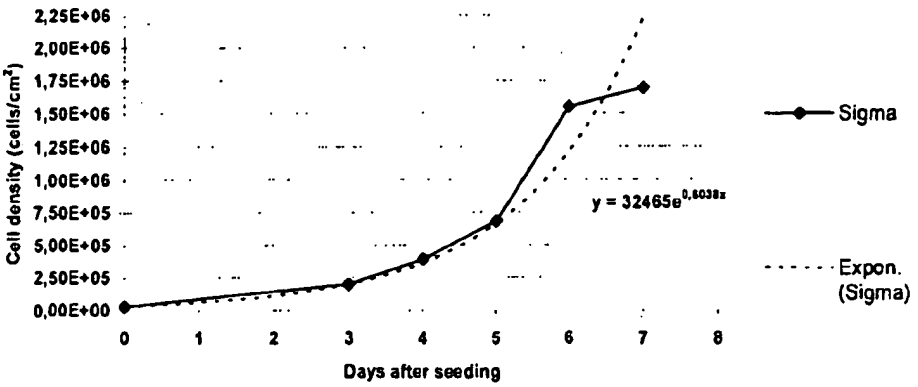
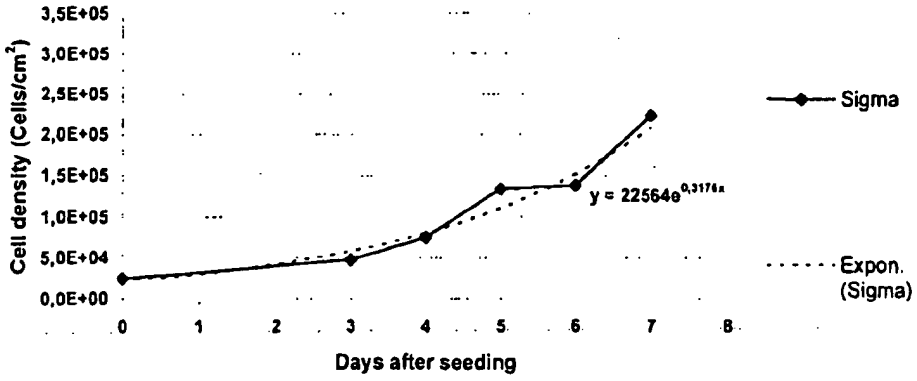


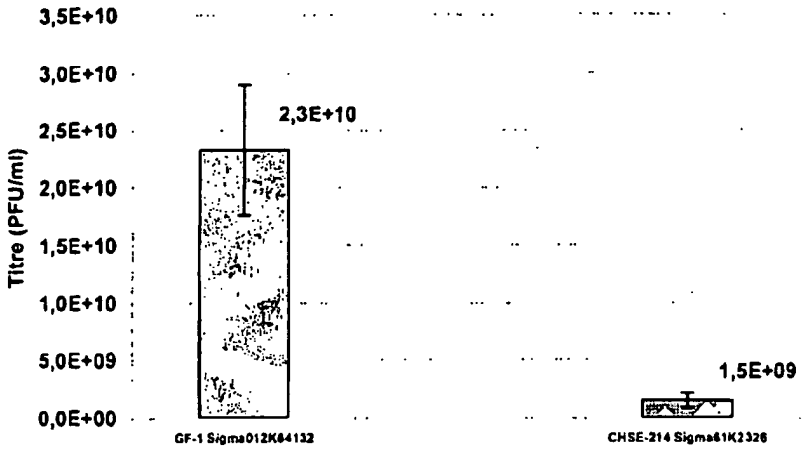

**Figure 2.** Cell growth promotion test on GF-1 cells with 75 $cm^2$ cell culture flasks. Two flasks were counted at each time point.




*(B) Test for IPNV yields*

The average IPNV titre for the CHSE-214 cells were 1.5 x $10^9$ PFU/ml (figure 3). The average IPNV titre for the GF-1 cells grown with serum were 2.3 x $10^{10}$ PFU/ml (figure 3).

**Figure 3.** Average IPNV titres in supernatants from GF-1 and CHSE-214 cell cultures and infected with IPNV.






5. It is my understanding, based on my knowledge and experience in the field of marine fish diseases, that grouper is not a susceptible host for IPNV so that IPNV would not propagate in grouper. On the other hand, it is well-known in the field that salmon is susceptible to IPNV. In fact, it has been found that IPNV caused major outbreak in salmonid species, including trout and salmon. Thus, I was astonished, based on the results of my comparative studies of IPNV titers in the GF-1 and CHSE-214 cell lines, that IPNV is actually propagated in a grouper cell line. I was even more surprised to find out that the titer of IPNV in the GF-1 cell line was more than 10 times higher that that in the CHSE-214 cell line, as salmon is known to be susceptible to IPNV and grouper is not.

I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true and further that these statements are made with the knowledge that willful false statements and the

like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

31 August 2005 — Date

Kjersti Gravingen — Kjersti Gravingen